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Quantification of Protein Signatures in Archived Human Prostate  
Tissues Using Shotgun Proteomic Methods

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14. ABSTRACT Biomarkers that robustly predict the metastatic potential of localized CaP are sorely needed to effectively treat localized CaP patients that pose the greatest risk of developing significant CaP. Biomarkers specific to significant CaP are also necessary if more effective drugs are going to be developed that can target and cure patients afflicted by this deadly disease. Proteins represent some of the most powerful molecular biomarkers to human disease such as cancer. Therefore this proposal will implement state-of-the-art methods in biological mass spectrometry to identify protein biomarkers specific to non-significant and significant CaP. These new protein biomarkers may spur the development of molecular tests that robustly predict the metastatic potential of non-significant CaP. These tests would reduce the physical and mental burdens associated with the overtreatment of patients afflicted by localized CaP. Also, protein biomarkers specific to significant CaP may represent new and effective drug targets to cure patients already afflicted by this deadly disease. We anticipate this proposal will identify the critical molecular targets with the greatest potential to improve the treatment and potentially cure CaP in men.					
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## INTRODUCTION

Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

My laboratory is currently constructing protein expression libraries of matched normal and cancerous human prostate tissues using quantitative mass spectrometry. Our research progress over the past 9 months has entailed the processing of formalin-fixed paraffin-embedded (FFPE) tissues of normal, T2, and T3 staged prostate tissues for proteomic analyses using label-free, quantitative mass spectrometry. We have acquired commercially available FFPE tissue blocks of whole prostate radical prostatectomies and have focused on optimizing protocols to extract and profile proteins in matched normal and diseased tissue samples using targeted proteomic methods. The ultimate outcome of these efforts will be the identification of pathologically-staged protein biomarkers to organ-confined and metastatic human prostate cancers.

## BODY

This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the Army Contracting Officer Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

**Task:** *Specific aim1: Construction of mass spectrometry-based protein expression libraries of normal prostate tissue, non-significant CaP tissue, and significant CaP tissue.*

My laboratory recently acquired 10 commercially available FFPE radical prostatectomy (RP) tissue blocks of matched normal and cancerous prostate tissues to build stage-specific protein expression signatures of non-significant (organ-confined) and significant (metastatic) human prostate cancer using label-free, quantitative mass spectrometry. In contrast to needle-biopsied FFPE tissue microarray samples, which routinely yielded 1-2 micrograms of total protein per sample (e.g. 5 micron x 2 mm needle-biopsy), while we have extracted up to 100 micrograms of total protein from the whole-mount FFPE RP tissue block samples. This has greatly enhanced our ability to interrogate the protein expression patterns in non-significant and significant human prostate cancers by nanospray liquid-chromatography tandem mass spectrometry (nano-LC-MS/MS). The FFPE tissue blocks provide up to 50X more material to identify human prostate cancer protein biomarkers.

My laboratory has also optimized a new protein-extraction protocol to interrogate the proteome of FFPE tissue samples by nano-LC-MS/MS. The optimized protocol involves the use of TCEP, SDS, and urea to efficiently extract formalin crosslinked proteins in paraffin-embedded tissues. We plan on publishing this optimized protocol shortly.

To expedite the discovery of pathologically-staged protein biomarkers to non-significant and significant human prostate cancer in FFPE RP tissue samples, my laboratory has also implemented a new mass spectrometry workflow called “Targeted Proteomics”(1). In contrast to shotgun proteomic methods, which seek to sequence all ionized tryptic peptides in a complex samples by nano-LC-MS/MS, we are utilizing a mass spectrometry profiling strategy to detect expression differences across multiple tissue samples. In contrast to randomly sequencing all peptides in the complex sample we have been able to selectively target and sequence differentially expressed peptide ions across multiple samples. The results of this targeted proteomic approach have been superior to our past shotgun proteomic experiments. This reflects our ability to sequence lower intensity peptide ions in the complex tissue sample relatively to shotgun proteomic protocols that routinely sequence high-abundant peptide ions using data-dependent (DD) acquisition methods of shotgun proteomic workflows. We will be submitting a manuscript in the next year to describe this new data-acquisition scheme to identify and quantify protein biomarkers in FFPE RP tissue samples shortly.

Moreover, my laboratory has established a new collaboration with Dr. Michael B. Cohen, the departmental head of Pathology at the UI Carver College School of Medicine. Dr. Cohen has provided pathological expertise in the annotation of commercially available FFPE RP tissue blocks. Dr. Cohen’s has volunteered his time to help my laboratory carefully annotate the Gleason score of non-significant and significant human prostate cancer samples.

Lastly, we have plans to start processing another 20 FFPE RP tissue samples in the next several months. These samples will allow us to build comprehensive protein expression libraries of normal prostate tissue, non-significant prostate cancer tissue, and significant prostate cancer tissue.

***Task: Specific aim 2: Identification of protein biomarkers in non-significant and significant CaP tissues using novel statistical methods.***

My laboratory has also been working with LabKey, the developers of Computational Portal Analysis System (CPAS) database, to develop plug-in statistical modules to identify statistically significant protein biomarkers of non-significant and significant human prostate cancer samples.

**KEY RESEARCH ACCOMPLISHMENTS:** Bulleted list of key research accomplishments emanating from this research.

- My laboratory is actively generating protein expression profiles of normal prostate tissue, non-significant prostate cancer tissue, and significant prostate cancer tissue by targeted proteomic methods.
- An optimized tissue-extraction protocol has been developed for the proteomic analysis of FFPE RP tissue samples.

- We have successfully implemented a targeted proteomic approach to identify and quantify protein expression changes across normal prostate tissue, non-significant prostate cancer, and significant human prostate cancer.

**REPORTABLE OUTCOMES:** Provide a list of reportable outcomes that have resulted from this research to include:

My laboratory is actively profiling samples at the moment. We have not had sufficient time to complete the proposed studies to date. We anticipate reportable outcomes over the next reporting period.

**CONCLUSION:** Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

The proposed research studies are ongoing. We have not reached any conclusions regarding the completed research to date. We anticipate over the next reporting period that distinct protein expression patterns will be obtained on normal prostate tissue, non-significant prostate cancer, and significant human prostate cancer. These findings will identify novel protein biomarkers to non-significant and significant human prostate cancer.

**REFERENCES:** List all references pertinent to the report using a standard journal format (i.e. format used in *Science*, *Military Medicine*, etc.).

1. Schmidt A, Claassen M, Aebersold R 2009 Directed mass spectrometry: towards hypothesis-driven proteomics. *Curr Opin Chem Biol* 13:510-517

**APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None reported to date.

**SUPPORTING DATA:** All figures and/or tables shall include legends and be clearly marked with figure/table numbers.

Data analyses are not complete. Completed datasets will be provided over the next reporting period.